(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:4:

ACCGTCCTTG ACACGATGGA CTCC

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i x) FEATURE:
(A) NAME/KEY: modified_base
(B) LOCATION: 6
(D) OTHER INFORMATION: /note= "U may be 5-[3- (alpha-iodoacetamido)-propyl]-2'-deoxyuridine"

(i x) FEATURE:
(A) NAME/KEY: modified_base
(B) LOCATION: 6
(D) OTHER INFORMATION: /note= "U may be 5-[3-(4- bromoburyramido)-propyl]-2'-deoxyuridine"

(i x) FEATURE:
(A) NAME/KEY: modified_base
(B) LOCATION: 6
(D) OTHER INFORMATION: /note= "U may be 5-[4- (alpha-iodoacetamido)-butyl]-2'-deoxyuridine"

(i x) FEATURE:
(A) NAME/KEY: modified_base
(B) LOCATION: 6
(D) OTHER INFORMATION: /note= "U may be 5-[4-(4- bromoburyramido)-butyl]-2'-deoxyuridine"

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:5:

CTCCAUCGTG TCAAG

What is claimed is:

1. An oligonucleotide having at least one nucleotide of the formula

$$R_1—B—(CH_2)_q—(Y)_r—(CH_2)_m—A'$$

wherein

R_1 is a 1-(β-D-ribofuranosyl) or 1-(β-D-2-deoxyribofuranosyl) group which is optionally substituted on one or more of its hydroxyl functions with a Z group, wherein Z independently is methyl or a phosphate, thiophosphate, alkylphosphate or alkanephosphonate group;

B is a heterocyclic base selected from purine and pyrazolo [3,4-d]pyrimidine groups wherein the $(CH_2)_q$ group is attached to the 7-position or 8 position of the purine and 3-position of the pyrazolo[3,4-d]pyrimidine groups and the R_1 group is attached to the 9-position of the purine and to the 1-position of the pyrazolo[3,4-d]pyrimidine groups;

Y is a functional linking group selected from a group consisting of —O—, —S—, —NR'—, —NH—CO—, trifluoroacetamido and phtalimido groups where R' is H or C_{1-6} alkyl, and at least one of the $(CH_2)_m$ and $(CH_2)_q$ groups is directly linked to the —O—, —S—

24

15

—NR'—, NH—CO—, trifluoroacetamido and phtalimido groups and the other of said $(CH_2)_m$ and $(CH_2)_q$ groups is linked to the heterocyclic base with a carbon to carbon bond;

m is 1 to 8, inclusive;

q is 0 to 8, inclusive;

r is 0 or 1; and

A' is a group selected from chloro, bromo, iodo, SO_2R'', $S^-R'^+R''$ and a radical which activates the carbon to which it is attached for nucleophilic substitution, where each of R'' and R'' is independently C_{1-6} alkyl or aryl or R'' and R'' together form a C_{1-6} alkylene bridge.

2. An oligonucleotide according to claim 1 wherein B is selected from adenine-8-yl, guanine-8-yl, 4-aminopyrazolo[3,4-d]pyrimidin-3-yl, and 4-amino-6-oxopyrazolo[3,4-d]pyrimidin-3-yl groups.

3. An oligonucleotide according to claim 1 wherein m is 1, 2 or 3; q is 2, 3, or 4; and r is 1.

4. An oligonucleotide according to claim 1 wherein the R_1 group is 1-(β-D-ribofuranosyl).

5. An oligonucleotide according to claim 1 wherein the R_1 group is 1-(β-D-2-deoxyribofuranosyl).

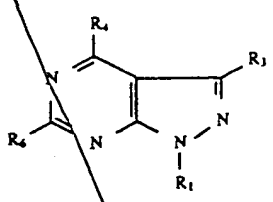
6. An oligonucleotide according to claim 1 wherein the R_1 group is 1-(β-D-2-O-methyl-ribofuranosyl).

7. An oligonucleotide according to claim 1 wherein the group $—(CH_2)_q—(Y)_r—(CH_2)_m—A'$ is

Sub. a1

3-iodoacetamidopropyl. 3-(4-bromobutyramido)propyl. 4-iodoacetamidobutyl. or 4-(4-bromobutyramido)butyl.

8. A compound of the formula



where R_1 is H. or a 1-(β-D-ribofuranosyl) or 1-(β-D-2-deoxyribofuranosyl) group which is optionally substituted on one or more of its hydroxyl functions with a Z group wherein Z independently is methyl or a phosphate. thiophosphate alkylphosphate or alkanephosphonate group. or a reactive precursor of said phosphate. thiophosphate. alkylphosphate or alkanephosphonate group which precursor is suitable for internucleotide bond formation;

R_3 is $(CH_2)_q$—(Y)$_r$—$(CH_2)_m$—A" where A" is a group selected from chloro. bromo. iodo. SO_2R'''. $S^+R'''R''''$ and a radical which activates the carbon to which it is attached for nucleophilic substitution. where each of R''' and R'''' is independently C_{1-6} alkyl or aryl or R''' and R'''' together form a C_{1-6} alkylene bridge. or A" is an intercalator group. a metal ion chelator or a reporter group;

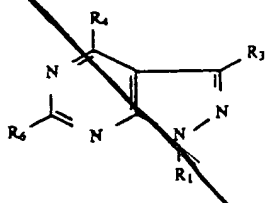
Y is a functional linking group selected from a group consisting of —O—. —S—. —NR'—. —NH—CO—. trifluoroacetamido and phtalimido groups where R' is H or C_{1-6} alkyl. and at least one of the $(CH_2)_m$ and $(CH_2)_q$ groups is directly linked to said —O—. —S—. —NR'—. NH—CO—. trifluoroacetamido and phtalimido groups and the other of said $(CH_2)_m$ and $(CH_2)_q$ groups is linked to the heterocyclic base with a carbon to carbon bond;

each of m and q is independently 0 to 8. inclusive; r is 0 or 1 provided that when A" is a group selected from chloro. bromo. iodo. SO_2R'''. $S^+R'''R''''$ and a radical which activates the carbon to which it is attached for nucleophilic substitution. then m is not 0;

each of R_4 and R_6 is independently H. OR. SR. NHOR. NH_2. or $NH(CH_2)_tNH_2$ where R is H or C_{1-6}alkyl and t is an integer from 0 to 12.

9. A compound in accordance with claim 8 where each of R_4 and R_6 is independently selected from a group consisting of H. OH and NH_2.

10. A compound of the formula



where R_1 is H. or a 1-(β-D-ribofuranosyl) or 1-(β-D-deoxyribofuranosyl) group which is optionally substituted on one or more of its hydroxyl functions with a Z group wherein Z independently is methyl or a phosphate. thiophosphate. alkylphosphate or alkanephosphonate group. or a reactive precursor of said

phosphate, thiophosphate, alkylphosphate or alkanephosphonate group which precursor is suitable for internucleotide bond formation;

R_3 is $(CH_2)_q$—(Y)$_r$—$(CH_2)_m$—A" and A" is a reporter group;

Y is a functional linking group selected from a group consisting of —O—, —S—, —NR'—, —NH—CO—, trifluoroacetamido and phtalimido groups where R' is H or C_{1-6} alkyl, and at least one of the $(CH_2)_m$ and $(CH_2)_q$ groups is directly linked to said —O—, —S—, —NR'—, NH—CO—, trifluoroacetamido and phtalimido groups and the other of said $(CH_2)_m$ and $(CH_2)_q$ groups is linked to the heterocyclic base with a carbon to carbon bond;

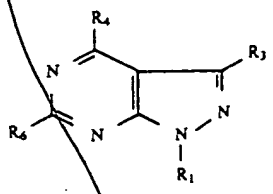
each of m and q is independently 0 to 8, inclusive; r is 0 or 1, and

each of R_4 and R6 is independently H, OR, SR, NHOR, NH_2, or $NH(CH_2)_tNH_2$ where R is H or C_{1-6}alkyl and t is an integer from 0 to 12.

11. A compound in accordance with claim 10 where each of R_4 and R_6 is independently selected from a group consisting of H, OH and NH_2.

12. A compound in accordance with claim 11 where the reporter group is biotin or 2,4-dinitrobenzene.

13. An oligonucleotide having at least one nucleotide of the formula



wherein R_1 is a 1-(β-D-ribofuranosyl) or 1-(β-D-2-deoxyribofuranosyl) group which is optionally substituted on one or more of its hydroxyl functions with a Z group wherein Z independently is methyl or a phosphate, thiophosphate, alkylphosphate or alkanephosphonate group;

R_3 is $(CH_2)_q$—(Y)$_r$—$(CH_2)_m$—A and A is a reporter group;

Y is a functional linking group selected from a group consisting of —O—, —S—, —NR'—, —NH—CO—, trifluoroacetamido and phtalimido groups where R' is H or C_{1-6} alkyl, and at least one of the $(CH_2)_m$ and $(CH_2)_q$ groups is directly linked to said —O—, —S—, —NR'—, NH—CO—, trifluoroacetamido and phtalimido groups and the other of said $(CH_2)_m$ and $(CH_2)_q$ groups is linked to the heterocyclic base with a carbon to carbon bond;

each of m and q is independently 0 to 8, inclusive; r is 0 or 1, and

each of R_4 and R6 is independently H, OR, SR, NHOR, NH_2, or $NH(CH_2)_tNH_2$ where R is H or C_{1-6}alkyl and t is an integer from 0 to 12.

14. An oligonucleotide in accordance with claim 13 where each of R_4 and R_6 is independently selected from a group consisting of H, OH and NH_2.

15. An oligonucleotide in accordance with claim 14 where the reporter group is biotin or 2,4-dinitrobenzene.

* * * * *

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[54] CROSS-LINKING OLIGONUCLEOTIDES

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[73] Assignee: EPOCH Pharmaceuticals, Inc.. Bothell, Wash.

[21] Appl. No.: 334,490

[22] Filed: Nov. 4, 1994

Related U.S. Application Data

[63] Continuation of Ser. No. 49,807, Apr. 20, 1993, abandoned, which is a continuation of Ser. No. 353,857, May 18, 1989, abandoned, which is a continuation-in-part of Ser. No. 250,474, Sep. 28, 1988, abandoned.

[51] Int. Cl.[6] C07H 19/04; C07H 21/00; C07H 21/02; C07H 21/04

[52] U.S. Cl. 536/26.7; 536/24.5

[58] Field of Search 536/26.1, 26.12, 536/26.13, 26.14, 26.8, 27.6, 27.81, 28.5, 28.54, 26.7, 24.5

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